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Summary

The objective of this study was to examine the impact of polymorphisms in the acyl-CoA:diacylglycerol acyltransferase (DGAT1), leptin and growth hormone receptor genes on body energy (body condition score, total body energy content and cumulative effective energy balance) and blood metabolic traits (levels of b-hydroxybutyrate, glucose and non-esterified fatty acids), measured once before the first calving and then repeatedly throughout first lactation in 497 Holstein cows. The influence of the same polymorphisms on cow reproductive performance and health during the first and second lactations was also assessed. Several reproductive traits were considered including interval, conception and insemination traits, as well as incidence of metritis and reproductive problems. Genotyping was performed using PCR-RFLP (DGAT1, leptin) or allele-specific PCR (growth hormone receptor). For each locus, the effect of allele substitution on body energy and blood metabolic traits was estimated using random regression models. The same effect on reproductive traits was assessed with single-trait mixed linear models. Significant ($P < 0.05$) effects on specific reproductive traits were observed. DGAT1 and growth hormone receptor alleles responsible for significant increases in milk production were found to have an adverse effect on reproduction, while the leptin allele responsible for significant increase in milk production was linked to marginally increased metritis frequency. Furthermore, the three studied loci were also found to significantly ($P < 0.05$) affect certain body energy and blood metabolic traits. Several associations are published for the first time, but these should be confirmed by other investigators before the polymorphisms are used in gene-assisted selection.

Key words: DGAT1, energy balance, gene, growth hormone receptor, leptin, reproduction.

Introduction

In the last decade, the availability of molecular biology techniques has provided conventional dairy cattle breeding and selection schemes with an additional tool: genomic data that can be used for gene- or marker-assisted selection. This kind of selection is based on the identification of genes or markers that may affect economically important traits of dairy cows. Several polymorphisms in various gene loci have been reported to affect production traits such as milk yield and composition. Polymorphisms in the acyl-CoA:diacylglycerol acyltransferase (DGAT1), leptin (LEP) and growth hormone receptor (GHR) gene loci are some notable examples.

The DGAT1 gene, which maps at the centromeric end of BTA14, is known to encode acyl-CoA:diacylglycerol acyltransferase, the enzyme that catalyses the last step in triglyceride synthesis. A non-conservative lysine-to-alanine substitution (p.Lys232Ala) in this gene has been proved to have a major influence on milk production traits and particularly on milk fat content (Grisart et al. 2002; Gautier et al. 2007). Ashwell et al. (2004) suggested possible pleiotropic effects of this polymorphism on reproduction (pregnancy rate) and Kaupe et al. (2007) provided some evidence supporting the assumption.

The LEP gene (BTA4) encodes leptin, a hormone that is involved in the regulation of feed intake, energy balance, fertility and metabolism (Fruhbeck et al. 1998; Macajova et al. 2004). Several polymorphisms in this gene have been studied for their association with economically important traits. A polymorphism in intron 2 of the LEP gene, first reported by Pomp et al. (1997), was found to affect milk production and feed intake (Liefers et al. 2002).

The GHR gene (BTA20) controls the function of the growth hormone receptor, which largely determines the action of the growth hormone. A phenylalanine-to-tyrosine substitution (p.Phe279Tyr) in this gene has been shown to affect milk yield and composition (Blott et al. 2003; Viitala et al. 2006).

Before any polymorphism information is used for the genetic improvement of dairy cattle productivity, their effect on other economically important traits should be examined. This way, potential unfavourable effects of the selected polymorphism on such traits because of pleiotropy or linkage could be avoided. Given the well-established unfavourable genetic correlation between milk production and reproduction in dairy cows (Berger et al. 1981; Dematawewa & Berger 1998; Windig et al. 2006), as well as the importance of reproductive efficiency in the economics of a dairy cow enterprise, the effect of these polymorphisms on reproductive traits should be investigated.

The investigation of the relationship of these polymorphisms with cow energy balance also seems justified because the adverse effect, which selection for milk production has on reproduction, can be mainly attributed to the increase, in both magnitude and duration, in the postpartum negative energy balance period (Lucy 2001). This period is characterized by inadequate daily energy intake with regard to essential cow functions such as maintenance, growth and milk production. For cows to carry out these functions, they mobilize energy reserves that could otherwise be used to sustain their reproductive performance. Monitoring daily changes in energy balance requires the recording of the daily feed intake of each cow, which, however, is not possible under field conditions. For this reason, other traits known to be related to energy balance can be used. Body condition scoring, a subjective but reliable and widely accepted way of assessing a cow_s body energy reserves (Edmonson et al. 1989; Fox et al. 1999), is an example of such a trait. Body condition score (BCS) and live weight records can be used for the calculation of total body energy content (EC) and cumulative effective energy balance (CEEB), which have also been proposed as energy balance indicator traits (Banos et al. 2006). Furthermore, various blood metabolic traits have been associated with the mobilization of a cow's energy reserves. For example, blood serum levels of glucose, β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) have been reported to be strongly correlated with energy balance (Reist et al. 2002; Clark et al. 2005).

The objective of this study was to examine the impact of the above-described polymorphisms in the DGAT1, LEP and GHR genes on body energy traits (BCS, EC, CEEB), blood metabolic traits (glucose, BHBA, NEFA) and reproductive traits in dairy cows.

Materials and Methods

Population description

The study was conducted in a large commercial dairy farm in Northern Greece (41 °02'37'N, 25 °15'16'E, altitude 20 m above sea level). Cows were housed in four free-stall barns and fed, twice daily, a total mixed ration to meet their energy and protein requirements. The ration formulation was based on US National Research Council recommendations (NRC, 2001). Four-hundred and ninety-seven (497) primiparous Holstein cows that calved at an average age of 842 ± 80 days between January 2005 and July 2006 were included in the study. These animals had either been born on the farm or had been imported as pregnant

heifers from three other European countries (Austria, France and the Netherlands). The latter is a rather common practice amongst many commercial dairy farms in Greece.

Cows were milked twice daily and their milk production was automatically recorded. Daily milk records were used for the calculation of 305-day yields. Pedigree information was available for all cows in the herd. After considering all animals related to the cows in this study, the total population size increased to 3306, spanning the three most recent generations. All these data were included in the subsequent analyses.

Body energy and blood metabolic traits

Cow BCS was assessed after the morning milking by a trained veterinarian on a weekly basis from calving to week 13 of lactation and thereafter, monthly, until the end of a 305-day lactation. A 5-point scale (1 = emaciated, 5 = obese; scored in 0.25-point intervals) and the method described by Ferguson et al. (1994) were used. At the same time, cow live weight was estimated using a heart girth tape (Webo) and blood sample was drawn from the coccygeal vein in a randomly selected subset (365 animals) of the
studied cows.

Blood samples were left to clot at room temperature for approximately 30 min and then centrifuged at 2000 g. The obtained serum samples were stored at)20 °C until analysed for glucose, BHBA and NEFA concentrations. Serum glucose and NEFA were assayed colorimetrically using commercial kits (Glucose GOD-PAP method, P. Zafiropoulos S.A. and Wako NEFA C kit, Wako Chemicals GmbH respectively) and a Hitachi U-2000 spectrophotometer. β-Hydroxybutyrate serum concentration was assayed using the same spectrophotometer and an enzymatic kinetic method based on the oxidation of BHBA to acetoacetate by BHBA dehydrogenase in the presence of NAD^+ (Bruss 1997).

The final dataset consisted of 8094 BCS, 8087 estimated live weight and 6015 serum glucose, BHBA and NEFA concentration records. BCS and estimated live weight records were used for the calculation of total body EC and CEEB with a procedure that is described by Banos et al. (2006). Table 1 shows descriptive statistics of all of these traits.

Single measurements of BCS, EC and serum levels of glucose, BHBA and NEFA, taken approximately 2 months before calving, were also available for a subgroup of the studied animals. Calculation of CEEB before calving was not possible because it related to energy changes between consecutive measurements and there was only a single record for each pregnant heifer. Descriptive statistics of these heifer traits are in Table 1.

Reproductive traits

Cows were observed for signs of oestrus twice daily for 30 min each, as well as during the morning and afternoon milking. Cows detected in oestrus were artificially inseminated by the same two experienced veterinarians 12 h later. Cows that did not exhibit oestrus within 60–80 days postpartum joined a combined gonadotropin-releasing hormone-prostaglandin oestrus synchronization programme, following the GPG protocol described by Stevenson et al. (1996). Pregnancy was diagnosed by rectal palpation 45–55 days after insemination. Cows with signs of metritis were diagnosed by the farms veterinarians and treated as appropriate.

All events regarding cow reproductive performance were systematically recorded. The following traits were derived from these records, spanning the time period from January 2005 to November 2007 (all traits refer to first lactation unless otherwise stated): conception rate (0/1) following first insemination (CONC_1AI1), conception rate (0/1) in the first 305 days of lactation (CONC_305), number of inseminations per conception (NINS), number of inseminations per conception for cows that conceived in the first 305 days of lactation (NINS_305), interval (days) from calving to conception for cows that conceived in the first 305 days of lactation (CAL_CONC_305), interval (days) between the cows first and second calving (CI), presence (0/1) of metritis (METR), presence (0/1) of reproductive problems (REPRO_PROB) and conception rate (0/1) following first insemination of second lactation (CONC_1AI2). In this study, the trait REPRO_PROB was defined as an index of two different reproductive problems with practical interest to the farmer (diagnosed metritis or failure to conceive within the first 305 days of lactation). Descriptive statistics for these traits are presented in Table 2. Information on some traits was not available for animals that were involuntarily culled. As the main reason that led to involuntary culling was lameness, all lameness incidences were recorded and included in the analysis.

Genotyping procedure

DNA was extracted from whole blood samples taken from a randomly selected subset (319) of the above-described 497 cows using the NucleoSpin Blood kit (Macherey-Nagel). The manufacturer's instructions were followed throughout. The DGAT1 and LEP gene polymorphisms were determined using PCR and RFLP according to previously described protocols (Liefers et al. 2002; Kaupe et al. 2004). To exclude any possibility of false genotyping, samples with ambiguous results were reanalysed using different RFLP conditions (e.g. increasing restriction enzyme concentrations, decreasing PCR product volumes in the digestion mix, increasing digestion times). The DGAT1 polymorphism was

p.Lys232Ala, coding a lysine-to-alanine substitution (Grisart et al. 2002). The LEP polymorphism was an intron 2 polymorphism first described by Pomp et al. (1997) and then studied by Liefers et al. (2002).

For the detection of the GHR polymorphism (a T-to-A substitution within exon 8), an allele-specific PCR (AS-PCR) was developed. The following primers, which amplify a 341-bp sequence of the GHR gene, were designed: (i) two allelespecific forward primers, designated as 4962-N (5`-GGGCTAGCAGTGACATTATT-3`) and 4962-M (5`-GGGCTAGCAG TGACATTATA-3`), anneal between nucleotides 4943–4962 of the GHR gene (AM161140) and detect the normal and the mutant allele respectively and (ii) a common reverse primer designated as 5283-R (5`–ACCTCTGGGTCCTGGAATAAA-3¢), which anneals between nucleotides 5263–5283 of the same sequence. PCR amplification was performed in 25-ll reaction mixtures containing 500 ng genomic DNA, 67 mM Tris–HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 1.5 mM $MgCl_2$, 200 lM deoxynucleoside triphosphates (dNTPs), 0.4 μM each primer (4962-N/5283-R or 4962-M/5283-R), and 1 U *Taq* DNA polymerase (SmarTaq; Dialat Ltd). The temperature cycling protocol on a TAKARA Thermal Cycler consisted of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 40 s. The cycling was repeated 35 times. Each PCR reaction was initiated with a 5-min denaturation at 94 °C and terminated with a 5-min extension at 72 °C.

All PCR and RFLP products were analysed by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and visualized on a UV transilluminator. Once the genotypes were determined, allelic frequencies at each gene locus were calculated by gene counting. Deviations from Hardy–Weinberg equilibrium were examined for each locus using chi-squared tests.

Statistical analysis

The impact of the DGAT1, LEP and GHR polymorphisms on first lactation BCS, EC, CEEB, glucose, BHBA and NEFA was estimated with the following random regression model that takes into account repeated measures on the same animal; each trait was analysed separately and each polymorphism was also fitted separately.

$$Y_{ijkmn} = YS_i + C_j + a_1 \cdot age + a_2 \cdot milk + a_3 \cdot pol \qquad (1)$$

$$+ \sum_{n=0}^{3} b_n P_n W_m + \sum_{n=0}^{3} c_{kn} P_n W_m + PE_k + e_{ijkmn}$$

where Y_{ijkmn} is the record of cow k in week of lactation m, YS_i is a fixed effect of year-season of calving i, Cj is the fixed effect of country of origin j, a1 is the linear regression coefficient on age at calving (age), a2 is the linear regression coefficient on 305-day milk yield (milk), a3 is the linear regression coefficient on polymorphism effect (pol), W_m is the week of lactation m, bn is the fixed regression coefficient on week of lactation, c_{kn} is the random regression coefficient on week of lactation associated with cow k (including all known pedigree, representing the polygenic residual effect), P_n is the nth orthogonal polynomial of week m (n = order of polynomial), PE_k is the random permanent environment effect associated with cow k and e_{ijkmn} is a random residual term.

In model 1, the polymorphism's allelic effect was described as 0, 1 or 2, in each case corresponding to the number of copies of the substitution allele. For example, the DGAT1 polymorphism includes the lysine (K) and alanine (A) variants. For this polymorphism, the variable in model 1 would be assigned values 0, 1 and 2 for cows with the KK, KA and AA variants respectively; A is equivalent to the allele-substitution effect at this gene locus at the observed frequency. Thus, the effect of allele substitution at each locus was estimated with this model.

Individual records, taken on pregnant heifers before calving, were analysed with a model similar to model 1 that included the effect of days to calving, but excluded the fixed and random regressions on week of lactation and the permanent environment. Model 2 was used to determine the impact of the same three polymorphisms on reproduction traits; each trait was analysed separately and each polymorphism was also fitted separately.

$$Y_{ijklm} = YS_i + G_j + S_k + L_l + a_1 \cdot age + a_2 \cdot milk + a3 \cdot pol + a4 \cdot dim + cow_m + e_{ijklm} \quad (2)$$

where Y_{ijklm} is the reproductive record of cow m, G_j is a fixed effect indicating whether the cow was included in an oestrus synchronization scheme (j = 0, 1), Sk is a fixed effect of season of first insemination k, Ll is a fixed effect indicating whether the cow was diagnosed with lameness at any time throughout lactation (l = 0, 1), a_4 is the linear regression coefficient on days in milk at first insemination (dim), cow_m is a random effect of cow m (including all known pedigree, representing the polygenic residual effect) and all other effects were as described in model 1.

Following preliminary analyses, only factors with a significant ($P < 0.05$) effect on a reproductive trait were included in each analysis. Thus, the oestrus synchronization effect (G) was included in the analysis of conception rate traits only; season of first insemination (S)

was included in the analysis of conception rate following first insemination (first and second lactation), number of inseminations per conception and interval from calving to conception and lameness (L) was included in the analysis of calving interval, reproductive problems and conception rate in the first 305 days of lactation. All other effects shown in model 2 were included in all analyses. The effect of 305-day milk yield was included to derive solutions for constant production level.

In model 2, the regression of interest was on the polymorphism, which, as in model 1, was defined by the allelesubstitution effect and was adjusted for all other effects in the model. For comparison purposes, the effect of the three polymorphisms on 305-day lactation milk yield was also estimated with a model similar to model 2. Although only a subset of cows (319) was genotyped, records from all cows were included in the analyses. The pedigree relationship matrix was used to link these data to the effects of the polymorphisms.

In the above models, each polymorphism was fitted separately in a series of sequential analyses. The null hypothesis was always that of no polymorphism effect on the trait of interest. Given the correlation among the traits studied here, truly independent tests were those of the three polymorphisms. A Bonferroni correction was implemented to account for multiple hypothesis testing with regard to these polymorphisms.

Results

Genotypic and allelic frequencies estimated for the three gene loci are presented in Table 3. Both the LEP and GHR loci were in Hardy–Weinberg equilibrium, but the DGAT1 locus was found to ($P < 0.05$) deviate significantly from this equilibrium. The effect of each gene locus on reproductive traits is shown in Table 4. Table 4 also shows the average effect of each of the three polymorphisms on body energy and blood metabolic traits measured during the whole first lactation as well as the same effect on traits measured in early lactation (first 4 weeks) only. In all cases, these are the effects of substituting one allele for the other at the corresponding locus.

Replacement of the lysine by the alanine variant at the DGAT1 locus was found to lead to an increase in NINS by 0.61 ± 0.22 ($P < 0.05$). This corresponds to 0.30 phenotypic standard deviations (SDp). The same substitution also led to a reduction in CONC_305 by 0.16 ± 0.05 (0.33 SDp, $P < 0.05$) and an increase in REPRO_PROB by 0.13 ± 0.05 (0.26 SDp, $P < 0.05$) (Table 4). These effects remained significant after the Bonferroni correction. As far as body energy and blood metabolic traits during the entire first lactation are concerned, the same substitution led to an average lactation increase in BCS by 0.10 ± 0.03 (0.25 SDp, $P < 0.05$),

in EC by 175.80 ± 61.05 MJ (0.20 SDp, P < 0.05) and in glucose blood levels by 1.67 ± 0.66 mg/dl (0.09 SDp, P < 0.05); all effects were still significant after the Bonferroni correction. An associated reduction in lactation NEFA levels by 0.016 ± 0.007 mmol/l (0.07 SDp, P<0.05) was not significant after the correction (Table 4). The effect of the DGAT1 polymorphism on early lactation (first 4 weeks), body energy and blood metabolic traits was quite similar to the effect on whole lactation, with the additional impact on CEEB (83.44 ± 35.76 MJ; 0.11 SDp, P < 0.05, significant post-Bonferroni correction) (Table 4). The DGAT1 polymorphism had no effect on body energy and blood metabolic traits measured on pregnant heifers. Finally, replacement of the lysine by the alanine variant at the DGAT1 locus was found to lead to an increase in the 305-day lactation milk yield by 363.4 ± 152.8 kg (0.25 SDp, P < 0.05).

Replacement of a copy of the A allele by the B allele at the LEP locus appeared to have a marginal effect (P = 0.05) on METR, leading to an incidence increase by 0.08 ± 0.04 (0.26 SDp). There was also a tendency for reduced CONC_ 1AI2, by 0.22 ± 0.12 (P < 0.10), but the effect did not attain statistical significance (Table 4). The LEP polymorphism effect on whole-lactation body energy or blood metabolic traits was not significantly different from zero (Table 4). There was an indication for an effect on early lactation body energy traits (Table 4), leading to a reduction in BCS (0.12 ± 0.05; 0.26 SDp, P < 0.05) and EC (230.60 ± 102.10 MJ; 0.25 SDp, P < 0.05), although neither effect maintained its significance after the Bonferroni correction. Additionally, the B allele was found to be responsible for a significant increase in glucose levels measured on heifers 2 months before calving (8.97 mg/dl ± 3.97, P < 0.05), although the effect of the polymorphism on milk yield was not significantly different from zero.

Finally, replacement of the phenylalanine variant by the tyrosine variant at the GHR locus led to an increase in NINS by 0.50 ± 0.25 (0.25 SDp, P < 0.05), a decrease in CONC_305 by 0.16 ± 0.06 (0.33 SDp, P < 0.05) and an increase in REPRO_PROB by 0.16 ± 0.06 (0.33 SDp, P < 0.05) (Table 4); the last two were still significant after the Bonferroni correction. Furthermore, this substitution was associated with an increase in whole-lactation CEEB by 110.10 ± 46.28 MJ (0.10 SDp, P < 0.05, significant post- Bonferroni correction) and a decrease in whole-lactation NEFA by 0.014 ± 0.008 (P < 0.10) (Table 4) as well as an increase in CEEB measured in the first 4 weeks of lactation by 89.00 ± 43.99 MJ (P < 0.05) (Table 4); the latter, however, was not significant after the Bonferroni correction. No significant impact of this GHR polymorphism on milk yield or on any body energy and blood metabolic trait measured on pregnant heifers was observed.

Discussion

Allelic frequencies at the DGAT1 locus estimated in this study were similar to frequencies reported in other studies of different Holstein populations. For example, the frequency of the allele encoding lysine (K) was previously reported to be 0.54 (Kaupe et al. 2007), 0.59 (Szyda & Komisarek 2007) and 0.60 (Spelman et al. 2002). However, there are substantial differences among genotypic frequencies reported in the literature. Kaupe et al. (2007) reported frequencies of KK, KA and AA to be 0.16, 0.51 and 0.33 respectively. These frequencies were 0.45, 0.26 and 0.27 in the study of Szyda & Komisarek (2007) and 0.38, 0.43 and 0.18 in the study of Spelman et al. (2002). Genotypic frequencies observed in this study were not similar to any of these frequencies because AA animals were not found in our study.

Allelic and genotypic frequencies reported in this study for the RFLP1 and p.Phe279Tyr polymorphisms at the LEP and GHR loci respectively were very similar to frequencies reported in previous studies (Liefers et al. 2002; Blott et al. 2003; Viitala et al. 2006). For example, Blott et al. (2003) reported genotypic frequencies for the GHR p.Phe279Tyr polymorphism to be 0.67, 0.31 and 0.02 for FF, FY and YY respectively. Liefers et al. (2002) reported that the frequencies for the LEP RFLP1 polymorphism were 0.813, 0.185 and 0.002 for AA, AB and BB respectively.

The alanine (A) variant of the DGAT1 p.Lys232Ala polymorphism, the B allele of the LEP RFLP1 polymorphism and tyrosine (Y) variant of the GHR p.Phe279Tyr polymorphism were reported in previous studies to be responsible for significant increases in milk production (Grisart et al. 2002; Liefers et al. 2002; Blott et al. 2003). The same significant effect of the DGAT1 polymorphism on milk yield was also found in this study, although the effect of the other two loci was not significant. Furthermore, in this study, all the above alleles were found to have an adverse effect on various reproductive traits. Specifically, the alleles encoding alanine and tyrosine at the DGAT1 and GHR loci respectively were associated with more inseminations needed per conception, reduced conception rate during lactation and increased incidence of reproductive problems, whereas the B allele at the LEP gene was linked to marginally increased metritis frequency. Especially with regard to DGAT1, the effect on reproductive traits was similar in size to the effect on milk production, which amounted to 0.25 phenotypic standard deviations.

There is very little published literature on the direct association of the three polymorphisms studied here with reproductive traits. Kaupe et al. (2007) reported a negative effect of the lysine (K) variant (DGAT1 p.Lys232Ala polymorphism) on maternal non-return rate, while

Liefers et al. (2002) found no significant effect of the LEP B allele (RFLP1 polymorphism) on commencement of luteal activity assessed by increased blood progesterone levels. None of these two traits per se was addressed in this study. The closest trait to the above analysed here was conception rate at first insemination, on which neither the DGAT1 polymorphism nor the LEP polymorphism appeared to have a significant effect. Although our results broadly corroborate those of Liefers et al. (2002), we acknowledge differences in the methodology and animal populations used as well as in the definitions of traits in the three studies. For example, Kaupe et al. (2007) used a granddaughter design where grandsires and sires were genotyped, but phenotypic observations were made on their granddaughters and daughters, whereas in our study, both genotypic and phenotypic information was recorded in the same animals. Furthermore, Liefers et al. (2002) assessed cow fertility as manifested by the commencement of luteal activity, whereas this study considered both interval and conception traits. There is certainly scope for further research on the relationship between these polymorphisms and reproduction before the former are used for the genetic improvement in dairy cow productivity with gene-assisted selection.

Studies regarding the effect of the three polymorphisms on body energy or blood metabolic traits are largely missing from the international literature. Results from this study indicate that all of these polymorphisms can significantly affect certain body energy or blood metabolic traits. The B allele of the LEP RFLP1 polymorphism that was found in a previous study to increase milk production and feed intake (Liefers et al. 2002) was associated here with compromised body condition and decreased EC during the first weeks of lactation. Given the unfavourable relationship between production levels and energy balance, this was an expected result. On the other hand, the alanine variant of the DGAT1 p.Lys232Ala polymorphism and the tyrosine variant of the GHR p.Phe279Tyr polymorphism that were reported to be associated with increased milk production in previous studies (Grisart et al. 2002; Blott et al. 2003) were found in this study to have a favourable effect on effective energy balance accumulating over the entire lactation. This result may oppose the established antagonistic correlation between milk production and body energy. However, it should be noted that both are quantitative traits whose expression is controlled by the action of and interaction among many gene loci. Hence, there is always the possibility of a single gene with a favourable effect on both traits.

Because body energy and blood metabolic traits have not been studied in this context before, validation of our results by independent studies would be beneficial. It is important, at this point, to emphasize the fact that all effects on the studied traits were estimated at constant

milk yield. Therefore, the true impact of the three polymorphisms on the traits in question was assessed. As body energy and reproduction are genetically related to production, estimated effects would have been different, especially for the DGAT1 polymorphism, without correction for milk yield.

In conclusion, results from this study indicate that three polymorphisms at the DGAT1 and GHR gene loci may significantly affect certain body energy, blood metabolic and reproduction traits of dairy cows. The LEP gene was found to affect body energy traits, while it was also linked to marginally increased metritis frequency. These results open up possibilities for cow breeding and improvement in geneassisted selection.

Acknowledgments

Georgios Karagiannis and Danae Zabouli are gratefully acknowledged for their contribution in the laboratory analyses. Funding for this research was made available from the General Secretariat for Research and Technology of the Greek Ministry of Development and VIVARTIA AVEE. The first author acknowledges financial support from the Greek State Scholarships Foundation.

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Legends

Tab.1 - Descriptive statistics for body condition score (BCS), energy content (EC), cumulative effective energy balance (CEEB), blood serum levels of glucose, β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) measured throughout first lactation and descriptive statistics of body energy (BCSh, ECh) and blood metabolic traits (glucoseh, BHBAh, NEFAh) measured on heifers before calving.

Tab.2 - Descriptive statistics for the number of inseminations per conception (NINS), number of inseminations per conception for cows diagnosed pregnant in the first 305 days of first lactation (NINS_305), interval from calving to conception for cows diagnosed pregnant in the first 305 days of first lactation (CAL_CONC_305), interval between first and second calving (CI), conception rate following first insemination of first lactation (CONC_1AI1), conception rate in the first 305 days of first lactation (CONC_305), presence of metritis (METR), presence of reproductive problems (REPRO_PROB) and conception rate following first insemination of second lactation (CONC_1AI2).

Tab.3 - Genotypic and allelic frequencies (%) in the three studied gene loci.

Tab.4 - Allele-substitution effect (a) on reproductive traits, on body energy and blood metabolic traits measured throughout first lactation and on body energy and blood metabolic traits measured during the first 4 weeks of first lactation.

Tab.1

Trait (units of measurement)	Records (n)	Cows/heifers (n)	Mean	SD	Minimum	Maximum
BCS (1–5)	8094	497	2.47	0.44	1.25	5.00
EC (MJ)	8087	497	4464.64	946.99	2328.90	9928.17
CEEB (MJ)	8087	497	-423.79	973.83	-3690.27	5984.08
Glucose (mg/dl)	6015	365	74.32	19.67	12.00	190.00
BHBA (mmol/l)	6015	365	0.79	0.28	0.19	4.42
NEFA (mmol/l)	6015	365	0.32	0.30	0.02	4.00
BCSh (1–5)	192	192	3.24	0.47	2.25	5.00
ECh (MJ)	143	143	5559.21	951.22	3751.59	7719.63
Glucoseh (mg/dl)	174	174	70.60	19.80	20.00	122.00
BHBAh (mmol/l)	175	175	0.55	0.19	0.24	1.57
NEFAh (mmol/l)	142	142	0.49	0.41	0.05	2.50

Tab.2

Trait (units of measurement)	No.of cows	Mean	SD	Minimum	Maximum
NINS	315	2.97	1.82	1	11
NINS_305	233	2.27	1.15	1	7
CAL_CONC_305 (days)	233	179.16	72.49	28	305
CI (days)	247	498.04	111.00	308	897
CONC_1AI1 (0/1)	375	0.19			
CONC_305 (0/1)	339	0.66			
METR (0/1)	497	0.09			
REPRO_PROB (0/1)	359	0.41			
CONC_1AI2 (0/1)	109	0.29			

Tab.3

		Variant[1]		Genotypic frequency			Allelic frequency	
Gene locus	Polymorphism	0	+	00	0+	++	0	+
DGAT1	P.Lys232Ala	K	A	0.24	0.76	0.00	0.62	0.38
Leptin	RFLP1	A	B	0.78	0.22	0.00	0.89	0.11
Growth hormone receptor	p.Phe279Tyr	F	Y	0.75	0.24	0.01	0.87	0.13

K, lysine variant; A, alanine variant; F, phenylalanine variant; Y, tyrosine variant.

A, B alleles as described by Liefers et al. (2002).

[1] Amino acids encoded by DGAT1 alleles are lysine (K) and alanine (A). Variants for leptin (A and B) are as described by Liefers et al. (2002). Amino acids encoded by growth hormone receptor alleles are phenylalanine (F) and tyrosine (Y).

Tab.4

Reproductive traits

	DGAT1 (substitution of A for K)			Leptin (substitution of B for A)			Growth hormone receptor (substitution of Y for F)		
Trait[1]	b	SE	p-value	b	SE	P-value	b	SE	p-value
NINS	0.61	0.22	0.01[2,3]	-0.24	0.28	0.39	0.50	0.25	0.04[2]
NINS_305	-0.15	0.15	0.31	-0.20	0.18	0.26	0.04	0.18	0.84
CALV_CONC_305	3.81	9.65	0.69	-11.00	11.96	0.36	2.19	12.00	0.86
CI	21.09	14.68	0.15	9.60	17.79	0.59	17.80	17.32	0.30
CONC_1AI1	-0.03	0.04	0.52	0.05	0.05	0.32	-0.02	0.05	0.68
CONC_305	-0.16	0.05	0.00[2,3]	0.01	0.07	0.94	-0.16	0.06	0.01[2,3]
REPRO_PROB	0.13	0.05	0.01[2,3]	0.05	0.07	0.48	0.16	0.06	0.01[2,3]
METRITIS	-0.01	0.03	0.68	0.08	0.04	0.05	0.00	0.03	0.93
CONC_1AI2	-0.04	0.10	0.72	-0.22	0.12	0.06	0.00	0.11	0.98

Body energy and blood metabolic traits measured throughout first lactation

	DGAT1 (substitution of A for K)			Leptin (substitution of B for A)			Growth hormone receptor (substitution of Y for F)		
Trait[1]	b	SE	p-value	b	SE	P-value	b	SE	p-value
BCS	0.10	0.03	0.00[2,3]	-0.03	0.04	0.43	0.04	0.04	0.29
EC	175.80	61.05	0.00[2,3]	-49.31	86.54	0.57	78.49	75.91	0.30
CEEB	33.21	37.78	0.38	1.69	52.71	0.97	110.10	46.28	0.02[2,3]
GLU	1.67	0.66	0.01[2,3]	0.16	0.85	0.85	-0.15	0.76	0.85
BHBA	-0.010	0.010	0.35	0.021	0.013	0.11	0.012	0.012	0.32
NEFA	-0.016	0.007	0.02[2]	-0.006	0.008	0.45	-0.014	0.008	0.07

Body energy and blood metabolic traits measured during the first 4 weeks of first lactation

	DGAT1 (substitution of A for K)			Leptin (substitution of B for A)			Growth hormone receptor (substitution of Y for F)		
Trait[1]	b	SE	p-value	b	SE	P-value	b	SE	p-value
BCS	0.08	0.03	0.02[2,3]	-0.12	0.05	0.02[2]	-0.03	0.04	0.50
EC	111.90	72.86	0,12	-230.6	102.10	0.02[2]	-13.73	90.60	0.88
CEEB	83.44	35.76	0.02[2,3]	-10.73	49.37	0.83	89.00	43.99	0.04[2]
GLU	1.75	1.22	0.15	0.02	1.68	0.99	0.70	1.50	0.64
BHBA	-0.021	0.029	0.47	-0.013	0.040	0.75	-0.020	0.036	0.57
NEFA	-0.091	0.030	0.00[2,3]	-0.025	0.042	0.56	-0.056	0.038	0.14

[1] Trait definitions are in Table 2.

[2] Significant ($P < 0.05$).

[3] Significant after Bonferroni correction.